Partial Purification and Some Properties of Bamboo Mosaic Virus

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ABSTRACT

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A rod-shaped virus measuring 490×15 nm was isolated from two species of bamboo, *Bambusa multiplex* and *B. vulgaris,* in Brasilia, Brazil. The virus could be transmitted mechanically to a cultivar of bamboo *(B. vulgaris* 'vittatu'), *Gomphrena globosa,* and *Chenopodium amaranticolor.* The infected bamboos originally were introduced from the state of São Paulo and a field survey of 1,268 bamboo plants in areas near Brasilia showed none of them was infected with the virus. Thermal inactivation point of this virus was between 65 and 70 C. The virus lost infectivity in crude sap of bamboo when diluted to 10^{-6}, but not to 10^{-5}. In extracts of *C. amaranticolor,* however, it was still infectious at a dilution of 10^{-7}. The virus was partially purified and a virus-specific antiserum was obtained. Agar gel diffusion method was used successfully to detect this long rod-shaped virus. The virus did not react with antisera to three potexviruses in microprecipitin- or agar gel diffusion tests. This virus, named bamboo mosaic virus, is probably an undescribed virus and should be placed in the potexvirus group.

During a routine survey of plant virus diseases in central Brazil in 1974, a virus was found inducing mosaic symptoms on two species of bamboo plants, *Bambusa multiplex* Raeusch and *B. vulgaris* Schrader, in Brasilia, Brazil. This paper reports the occurrence, mechanical transmission, physical properties in vitro, partial purification, morphology, and serology of this virus, named bamboo mosaic virus (BoMV). Apparently, this is the first virus to be identified infecting bamboo plants. Some of the results were presented previously (4, 8).

MATERIALS AND METHODS

Mechanical transmission. — Inocula for the mechanical transmission trials were juices expressed from infected leaves in one of the following solutions: (A) 0.1 M phosphate buffer at pH 7.6; (B) 0.1 M citrate buffer containing 0.1% Na_2SO_3 at neutral pH; or (C) a (1:1, v/v) mixture of 0.1% each of Na_2SO_3 and bentonite (13). The inocula were rubbed by finger on Carborundum-dusted leaves of the test plants, or alternatively, a small amount of Celite (diatomaceous earth, Sigma Chemical Co., St. Louis, MO 63178) was added to the inoculum before it was rubbed on the leaves. All test plants were grown in 20.3-cm (8-inch) diam aluminum pots inside a screen house. Noninoculated plants were maintained as controls under similar conditions.

Partial purification. — Bamboo leaves with mosaic symptoms were cut into small pieces about 2 cm in length, ground with a chilled meat grinder, then ground in a blender in five volumes of 0.1 M or 0.2 M citrate buffer at pH 7.0 containing 0.1% Na_2SO_3 (and occasionally 0.1%

bentonite) and 0.01 M $MgCl_2$. The homogenates were strained through two layers of cheesecloth and clarified either by passing through a Celite pad (7) or by mixing with an equal volume of cold chloroform followed by low-speed centrifugation. The virus was precipitated from the clarified sap by addition of polyethylene glycol (M. W. 6,000) and NaCl to make up 8% and 0.05 M, respectively, and the viral precipitate was collected by low-speed centrifugation. The pellets were suspended in a small amount of 0.02 M phosphate buffer at neutral pH and clarified by low-speed centrifugation. The partially-purified virus preparations thus obtained were examined by electron microscopy and in a spectrophotometer and were also used as antigen to produce antiserum. Concentration of the virus preparations was estimated by measuring absorbance at 260 nm assuming an extinction coefficient of 3/mg/ml/cm for this virus.

Electron microscopy. — Particle morphology was studied either in leaf-dip preparations from BoMV-infected test plants or in partially-purified preparations stained with 3% uranyl acetate. Histological work was done with double-fixed (3% glutaraldehyde + 1% OsO_4) material embedded in Epon #812. The electron microscope used was a Zeiss EM 9.

Serology. — The antiserum to BoMV was prepared by injecting 2.2 mg of virus intravenously followed by two intramuscular injections of 3.3 mg each at 5 and 21 days after the first injection. An equal volume of Freund's complete adjuvant (Difco Lab., Detroit, MI 48232) was mixed with virus preparation for the intramuscular injections. The rabbit was bled by heart puncture at every 5-35 day intervals to test the titer. Antiserum was preserved in an equal volume of glycerin in a freezer. Serological tests were done by agar gel double diffusion

using 0.75% Special Noble agar (Difco) and by microprecipitin tests (7). Lyophilized antisera to papaya mosaic virus (PMV), clover yellow mosaic virus (CYMV), and potato virus X subunit protein (PVX-SP) supplied by D. E. Purcifull were dissolved in 0.85% NaCl to the original volumes. A solution of 0.85% NaCl was used as the diluent for antisera.

RESULTS AND DISCUSSION

Natural occurrence of the disease. — Among seven species of bamboo introduced from Instituto Agronomico, Campinas, São Paulo in 1970 and grown at the Biological Station of the University of Brasilia, two of them, *Bambusa multiplex* and *B. vulgaris,* were first noticed to have mosaic symptom on the leaves (Fig. 1-A) in 1974. Electron microscopic examination of the leaf-dip preparations indicated the association of flexuous rod particles (Fig. 1-D) with the mosaic. At the station, bamboo plants of each species were grown together as a bush and the seven species were planted in a row approximately 1 m apart. Only one single plant out of 66 *B. vulgaris* was infected with the mosaic, whereas all 30 plants of *B. multiplex* were infected. During a 3-yr period, the 65 *B. vulgaris* plants did not become infected although their leaves were in close contact with those of the infected one.

Compared with healthy plants, the infected bamboos showed no external symptom other than the mosaic syndrome. No information was available as to whether these plants were infected before or after they were brought to Brasilia. No weeds or other kinds of plants which carry the virus were found in the field near the infected bamboos at the station. A field survey made in the Federal District (which includes Brasilia) and the neighboring states of Goias and Minas Gerais during 1974-1975 showed that none of the 1,268 bamboo plants inspected, mostly *B. vulgaris* 'vittatu', was infected with the virus.

Mechanical transmission. — Bamboo mosaic virus could be transmitted mechanically to three species of plants by preparing the inoculum with either solution B or C, but not A. They were: one cultivar of bamboo (*B. vulgaris* 'vittatu'*)* which developed systemic mosaic, and *Gomphrena globosa* L. and *Chenopodium amaranticolor* Coste & Reyn.; both of them developed necrotic local lesions. The mosaic on leaves of bamboo began to show within 45 days after inoculation. Only the terminal leaves of certain branches showed the symptom. After the mosaic leaves were removed, the newly-formed terminal leaves exibited the mosaic symptom but the other leaves on the same branch remained symptomless during the 8-mo trial period. The inoculated *G. globosa* and *C. amaranticolor* began to show necrotic lesions (Fig. 1-B) and necrotic rings (Fig. 1-C), respectively, within 10 days after inoculation. The presence of virus particles in the tissues of lesion areas was confirmed by electron microscopic examination of leaf-dip preparations and ultrathin sections of infected tissues. The local lesion reaction in *C. amaranticolor* appeared to be more consistent and thus was used in later experiments to assay for the presence of BoMV.

The plants that did not show symptoms and from which BoMV could not be recovered were: *Chenopodium quinoa* Willd., *Phaseolus vulgaris* L. 'Manteiga', *Datura stramonium* L., *Nicotiana tabacum* L. 'Turkish', *N. glutinosa* L., *Lycopersicon esculentum* Mill., *Malva parviflora* L., *Oryza sativa* L., *Zea mays* L., *Capsicum annuum* L., *Lactuca sativa* L. 'E. Tower', *Pennisetum* sp., *Brassica oleracea* L. 'Gongarsol', *Raphanus sativus* L., *Vigna sinensis* (Torner) Savi., and *Cucurbita pepo* L.

Physical properties in vitro. — Inocula prepared from bamboo lost infectivity after being diluted to 10^{-6} but not to 10^{-5}, whereas that prepared from infected *C. amaranticolor* still was infectious after being diluted to 10^{-7}, the maximal dilution that was tested. The latter inoculum at 1/50 dilution lost infectivity after treatment for 10 min at 70 C but not 65 C. When stored at 8 C for 78 hr, most of the infectivity was retained.

Partial purification. — Partially-purified preparations of BoMV were infectious and appeared to be relatively free from contamination as evidenced by electron microscopic observation (Fig. 1-E). Virus particles in sap clarified by the Celite pad filtration tended to aggregate more than those clarified by chloroform. However, there was no significant difference in the purity and yield of the virus preparations after clarification by either method. The time allowed to precipitate the virus particles after addition of polyethylene glycol apparently was critical in purifying BoMV; when left in the refrigerator (5-8 C) for 2 hr, very few virus particles were recovered in the precipitate, but if left overnight, a fluffy white precipitate could be seen on the bottom of the flasks and large amounts of virus could be obtained. Very little host debris was precipitated with the virus. In routine preparations, about 19-25 mg of virus could be recovered from 100 g of infected bamboo leaves.

Ultraviolet absorption spectrum. — Preparations of BoMV had a peak absorption at 265 nm (Fig. 2). The absorption ratio, A $_{280/260}$ was 0.89, which indicated a nucleic acid content of about 4-5% (6).

Electron microscopy. — In leaf-dip preparations, the normal length (based on measurement of 205 particles) was 490 nm (Fig. 3). The particle width was 15 nm. In partially-purified preparations, BoMV particles were more heterogeneous in length (Fig. 1-E) probably as a result of breakage. No ultrastructural details such as an axial channel or subunits were observed. Aggregates of fibrous material were consistently noted in the cytoplasm and vacuoles of most of the parenchyma and vascular cells from mosaic-affected bamboo leaves and also from tissues of local lesions in *C. amaranticolor* and *G. globosa* leaves. The details of the histological localization of BoMV and its cytopathological effects are reported separately (5).

Serology. — The titer of the antiserum was determined by microprecipitin test. It increased rapidly from 1/30 on the day when the last injection was made to 1/240 in 5 days, 1/960 in 26 days, and reached a maximum of 1/7,680 in 34 days and then dropped to 1/1,920 12 days later. Reasonably high titer (1/240-1/480) was maintained until 4 mo after the last injection.

In agar gel diffusion tests, the antiserum reacted with crude saps prepared from leaves of bamboo or *C. amaranticolor* infected with BoMV or partially-purified BoMV preparations forming one major precipitin line near the antigen well and occasionally another faint line near the antiserum well. No line was observed when

normal serum was used or when the antiserum was reacted with preparations from healthy leaves of both plants. The formation of the major line could be due to the precipitation of intact BoMV particles by the homologous antibody because of the following observations; (i) The location of the line indicated the low diffusibility and
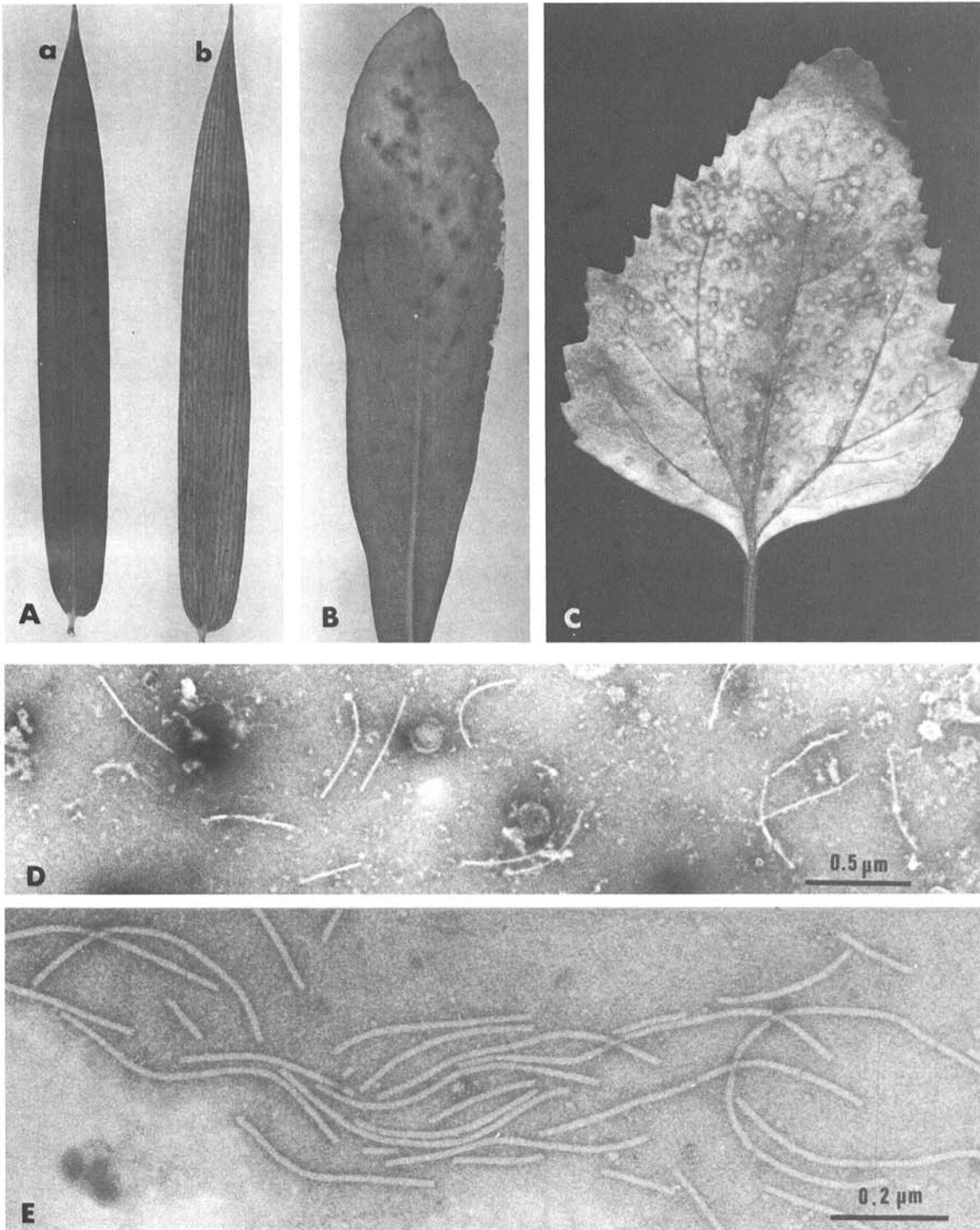

Fig. 1-(A to E). Symptoms incited by bamboo mosaic virus (BoMV) on leaves of **A-b)** *Bambusa vulgaris,* **B)** *Gomphrena globosa,* and **C)** *Chenopodium amaranticolor* compared with **A-a)** a healthy leaf of *B. vulgaris.* **D)** Leaf-dip preparation showing rod-shaped particles of BoMV. **E)** A partially-purified preparation of BoMV stained with 3% uranyl acetate.

thus the large size of the antigen. (ii) An antiserum preparation with a titer of 1/240 in agar gel diffusion tests had the same titer in microprecipitin tests and the precipitates had the amorphous, cloud-like appearance indicating the antigen was a long rod (1). (iii) When centrifuged in a gradient consisting of 4, 7, 7, and 7 ml of 10, 20, 30, and 40% sucrose in Beckman SW 25.1 rotor at 22,500 rpm for 2 hr, BoMV preparations gave an opalescent band 1.2 cm below the meniscus (versus 2.0 cm for TMV). The band, which contained exclusively BoMV particles as revealed by electron microscopy, was immediately removed and tested serologically. The same results as observation 2 were obtained. Lack of succcess of the agar gel diffusion method with long rod-shaped viruses

such as PVX and PVS has been noted by several workers. (11, 12). The ability of BoMV particles to diffuse in agar gel is unusual. This might be due to the lack of particle aggregation as observed during the purification and density gradient centrifugation.

To test the degree of relationship between the antiserum and degraded protein of BoMV, a partially-purified BoMV preparation (1 mg/ml) was treated with an equal volume of either 1% sodium lauryl sulfate or 0.2 M ethanolamine at pH 10.5 for 24 hr at 8-10 C and then reacted with serial dilutions of an antiserum which had a titer of 1/240 to the whole virus in agar gel plates. Both preparations of degraded protein formed a faint line near the antiserum wells with dilutions of up to 1/10. This suggested that there was difference in the antigenic identity between BoMV and its degraded protein as in the case of PVX (9).

A BoMV preparation with a concentration of 0.5 mg/ml did not react with the antisera to PMV, CYMV, or PVX-SP, either nondiluted or serially diluted to 1/60, in microprecipitin or agar gel diffusion tests. Members of the potexvirus group exist which have a distant serological relationship (3); however, the use of many high-titered antisera from different animals sometimes is required to establish distant relations (2).

Consideration of BoMV as a new virus. — All of the rod-shaped plant viruses known to have the same class of particle normal length as BoMV are classified as members or possible members of potexvirus group (3). None of these viruses is known to infect bamboo and has biological and serological properties identical to BoMV. Furthermore, no known potexviruses induce crystalline inclusions in cells of *C. amaranticolor* and *G. globosa* as did BoMV (5). Based on these data, BoMV is probably a hitherto undescribed plant virus. An unidentified virus was reported to cause mosaic in bamboo in Hawaii (10). Since no other information was given in that report, we do not know whether it is the same virus as BoMV.

Inclusion of BoMV in potexvirus group. — In aspects of particle morphology, infectivity in vitro, concentration of virus in leaf tissues, and nucleic acid content of virus particle, BoMV closely resembles members of potexvirus and previously was placed as a possible member of that group (3). The finding that the general pattern of cell infection by BoMV follows that exhibited by some potexviruses (5) provides further evidence. However, final statement that BoMV is a member of potexvirus group can only be made after thorough comparison of the physico-chemical properties and serological relationship of this virus with those of established members.

General remarks. — The bamboos are large perennial, woody grass plants. They are indigenous to southeastern Asia and are widely grown in tropical and subtropical regions throughout the world. In Brazil, bamboos are planted for wind-breaking and landscaping purposes. However, bamboo's stiff, hollow stems are used for making canes, furnitures, musical instruments, and even houses. In southeastern Asia, bamboo pulp is used to make high quality paper and bamboo shoot is a delicacy in oriental cooking. Rhizomes of bamboo are reported to have antihemorrhagic and antihemorrhoidal actions.

The only visual symptom in bamboo plants infected by BoMV was mosaic in leaves. The effects of this virus on the growth and production of stem, pulp, shoot, and
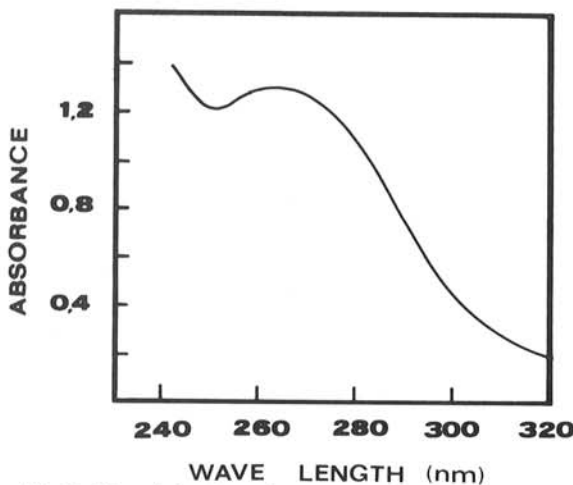

Fig. 2. Ultra-violet absorption spectrum of a partially purified preparation of bamboo mosaic virus.
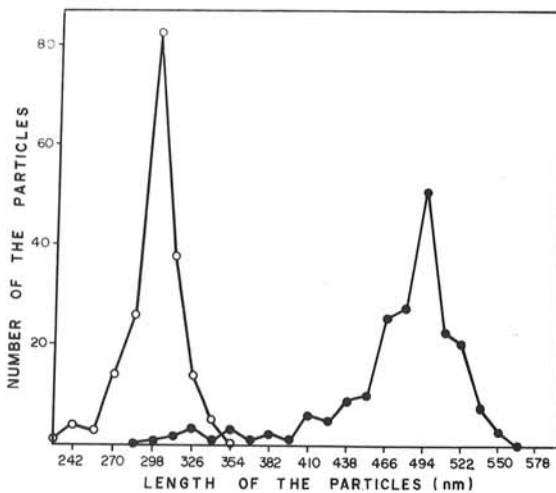

Fig. 3. Particle-length distribution of bamboo mosaic virus (•——•) and tobacco mosaic virus (o——o) in leaf dip preparations.

rhizome of bamboo are yet to be determined.

The BoMV-infected bamboo were originally introduced from the state of São Paulo. Since bamboo plants are propagated by vegetative means, we would expect the existence of the virus in that state. It is not known whether BoMV occurs in other parts of Brazil and in other bamboo-growing countries. The antiserum to BoMV prepared in this study should facilitate greatly a world wide survey on this disease.

Members of potexvirus group do not have known insect vector (3) and BoMV was mechanically transmissible with diffuculty, the only possible way of natural spread of this disease would be the use of cuttings from diseased plants. Thus, the control of this disease should be relatively simple.

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